Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2011.04409.x

In vitro effect of anti-human immunodeficiency virus CCR5 antagonist maraviroc on chemotactic activity of monocytes, macrophages and dendritic cells

R. Rossi,*‡ M. Lichtner,*†‡
A. De Rosa,* I. Sauzullo,*
F. Mengoni,* A. P. Massetti,*
C. M. Mastroianni*† and V. Vullo*
*Department of Public Health and Infectious Diseases, Istituto Pasteur-Fondazione Cenci Bolognetti, 'Sapienza' University, Rome, and †Infectious Diseases Unit, 'Sapienza' University, S. M. Goretti Hospital, Latina, Italy

Accepted for publication 28 March 2011
Correspondence: C. M. Mastroianni, Infectious Diseases Unit, Sapienza University, Polo Pontino, S. M. Goretti Hospital, Via Canova, 04100 Latina, Italy.
E-mail: claudio.mastroianni@uniroma1.it

‡R. Rossi and M. Lichtner contributed equally to this study.
E-mail address for senior author: vincenzo.vullo@uniroma1.it.

Summary

Compounds targeting the chemokine receptor CCR5 have recently been approved for treatment of human immunodeficiency virus (HIV) infection. Given the central role of CCR5 in inflammation and recruitment of antigen-presenting cells (APC), it is important to investigate the immunological consequences of pharmacological inhibition of CCR5. We evaluated the *in vitro* effect of different concentrations of CCR5 antagonist maraviroc (MVC) on cell migration of monocytes, macrophages (MO) and monocyte-derived dendritic cells (MDC) towards peptide formyl-methionyl-leucyl-phenylalanine (fMLP) and chemokines regulated upon activation normal T cell expressed and secreted (RANTES) and CCL4/macrophage inflammatory protein-1 (MIP-1β) and CCL2/monocyte chemotactic protein-1 (MCP-1). Results of flow cytometric analysis showed that monocytes treated *in vitro* with MVC exhibited a significant dose-dependent reduction of chemotaxis towards MIP-1β and MCP-1. fMLP-induced chemotactic activity decreased only at higher concentration (1 μM and 10 μM of MVC). In addition, all concentrations of MVC (0·1, 1 and 10 μM) induced *in vitro* a significant inhibition of chemotaxis of MO and MDC in response to all tested chemoattractants. No change in phenotype (CD1a and CD14) and CCR1, CCR4, CCR5 and formyl peptide receptor (FPR) expression was seen after *in vitro* treatment with MVC. These findings suggest that CCR5 antagonist MVC may have the *in vitro* ability of inhibiting the migration of innate immune cells by mechanism which could be independent from the pure anti-HIV effect. The drug might have a potential role in the down-regulation of HIV-associated chronic inflammation by blocking the recirculation and trafficking of MO and MDC.

Keywords: CCR5, chemotaxis, dendritic cells, HIV, maraviroc

Introduction

Antigen-presenting cells (APC), such as monocytes, macrophages and dendritic cells (DC), are important components in linking innate and adaptive immunity. The chemotactic recruitment of these cells at the site of infection is critical for the initiation of appropriate immune responses [1]. This migration is a complex, multi-step process, mediated by chemokines and their receptors. There are several data suggesting that chemokine receptor CCR5 is involved in both positive and negative regulation of the APC system by the modulation of leucocyte trafficking, cellular activation and cytokine expression [2]. Recently, compounds targeting CCR5 have been introduced into clinical practice for the treatment of human immunodeficiency virus (HIV) infection [3]. These drugs specifically inhibit the replication of R5-tropic HIV variants by blocking the interaction between the virus and the chemokine receptor CCR5, which is necessary for R5-using HIV strains to enter host cells [4,5]. However, the *in vitro* and *in vivo* immunological consequences of pharmacological inhibition of CCR5 function remain to be investigated. The greatest beneficial effects of maraviroc (MVC), the first approved CCR5 inhibitor, are well documented by clinical trials analysis [6,7]. In particular, the drug induces a greater immunological benefit that is independent of HIV load suppression. Various mechanisms could be involved in this phenomenon, such as down-regulation of excessive immune activation by CCR5 blockade, reduction of T cell apoptosis and cytokine expression. Considering the important role of CCR5 in both trafficking

© 2011 The Authors
Clinical and Experimental Immunology © 2011 British Society for Immunology, *Clinical and Experimental Immunology*

and recruitment of leucocytes, the analysis of the effect of CCR5 antagonists on the modulation of cell migration needs to be clarified.

In the present study, we assessed the direct *in vitro* effect of anti-HIV CCR5 antagonist MVC on chemotactic activity of human monocytes, macrophages (MO) and monocyte-derived DC (MDC) towards different chemoattractants. Chemotaxis receptor expression was also evaluated.

Materials and methods

Monocytes, MO and MDC

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors' buffy coat using density gradient centrifugation Ficoll-Histopaque (Gibco/BRL, Cergy Pontoise, France). PBMCs were collected, washed twice with phosphate-buffered saline (PBS) and incubated at a concentration of 1×10^6/ml in complete RPMI-1640 containing 10% fetal calf serum (FCS) and allowed to adhere for 2 h at 37°C in CO_2 5%. After incubation, non-adherent cells were removed and adherent cells were harvested and counted. When the cell preparation showed $\geq$ 90% CD14 expression, the generation of MO and MDC was carried out. Briefly, cells were cultured in RPMI-1640 supplemented with 10% FCS and glutamine (2 mM); granulocyte–macrophage colony-stimulating factor (GM-CSF) (50 ng/ml) (Leukomax, Schering-Plough, Dardilly, France) and interleukin (IL)-4 (40 ng/ml) (Peprotech, Rocky Hill, NJ, USA) were added for MDC generation, while G-CSF (50 ng/ml) was used for MO generation. After 5 days cells were tested for phenotype and maturation markers. Cell viability, characterization and maturation were assessed during the cell production process by light microscopy and flow cytometry using monoclonal antibodies CD1a-phycoerythrin (PE), CD14-fluorescein isothiocyanate (FITC), CD83-PE and CD86-FITC (BD, Becton Dickinson Europe, Pont-de-Claix, France). Viable cell preparations with a positivity higher than 95% for the specific markers were considered valid for subsequent analysis.

Drug treatment of cells

MVC (Celsentri; Pfizer, Inc., New York, NY, USA) was dissolved in distilled water and stored at −80°C until use. Monocytes, MO and MDCs (1×10^6/ml) were pre-incubated for different times (1–18 h) with various concentrations of MVC (0·1 μM, 1 μM, 10 μM) at 37°C under 5% CO_2 atmosphere. Because, in preliminary experiments, we found no differences in incubation time, we reported the data obtained from 18 h of MVC treatment. As controls, cells were incubated with medium alone.

Drug concentrations were chosen on the basis of published data of pharmacokinetic parameters reported in MVC-treated patients [8,9]. MVC-treated cells at all concentrations used showed a viability $\geq$ 95%, as assessed by Trypan blue exclusion dye.

Chemotaxis assay

The *in vitro* chemotactic activity was measured in an 8 μm pore size Transwell system (Becton Dickinson Europe). The following chemoattractants were used: synthetic peptide formyl-methionyl-leucyl-phenylalanine (fMLP) (10^{-5} M) (Sigma, St Louis, MO, USA), CCL5/regulated upon activation, normal T cell expressed and secreted (RANTES) (100 ng/ml), CCL4/macrophage inflammatory protein-1 (MIP-1β) (100 nM) and CCL2/monocyte chemotactic protein-1 (MCP-1) (10 ng) (R&D Systems Europe Ltd, Abingdon, UK). A bell-shaped curve described the typical migratory response of cells to increasing concentrations of chemoattractant. Thus, in preliminary experiments, we performed a full dose–response analysis and we used the optimal doses able to induce the maximum chemotactic activity in our cell systems.

Cell suspensions in FCS-free RPMI-1640 were used at a concentration of 1×10^6 cells/ml. After 30 min of incubation at 37°C in 5% CO_2 in a humidified atmosphere, the migrated cells in the lower well were quantified by flow cytometry [fluorescence activated cell sorter (FACS)Calibur with CellQuest software] using Trucount™ tubes (Becton Dickinson Europe). To eliminate cellular debris, R1 gate was defined in a dot-plot of forward-scatter channel (FSC) *versus* side-scatter channel (SSC). Random migration in the absence of chemoattractant was calculated and subtracted from migration in response to stimuli. Results were expressed as mean [±standard deviation (s.d.)] percentage of chemotaxis of six different experiments using different donors. Control chemotaxis was set at 100% and MVC treatments were represented as the percentage of control (cells incubated with medium alone). To confirm the data, the measurement of cell chemotaxis in some experiments was also carried out using Boyden's method with blind-well chambers and Diff-Quik staining of the filter (Baxter Diagnostics AG, Dudingen, Switzerland).

Analysis of the expression of chemoattractant receptors

The expression of chemokine receptors CCR1, CCR4, CCR5 and formyl peptide receptor (FPR) that recognize the three receptors for fMLP (FPR, FPR1, FPR2) was determined by flow cytometric analysis of MVC-treated monocytes, MO and MDC. Cells (1×10^5) were stained with CCR5-FITC/FPR-PE (Becton Dickinson Europe) and CCR1-PE/CCR4-FITC (R&D Systems). After 30 min of incubation, cells were washed with buffer (PBS, 2% FCS), fixed with 1% paraformaldehyde (PFA) and analysed using FACSCalibur with a minimum acquisition of 10 000 events. Differences in mean fluorescence intensity (MFI) between MVC-treated and -untreated cells were analysed with CellQuest software.

© 2011 The Authors

Clinical and Experimental Immunology © 2011 British Society for Immunology, *Clinical and Experimental Immunology*

Statistical analysis

SPSS version 13·0 for windows (SPSS Inc., Apache Software Foundation, Chicago, IL, USA) was used. Student's *t*-test was used for statistical analysis of chemotaxis.

Results

Analysis of MO chemotaxis following MVC *in vitro* treatment

MO were treated *in vitro* with increased concentrations of MVC and then examined for chemotaxis by cytometric evaluation (Table 1). No differences were found in the results, showing that pretreated MO did not exhibit a significant inhibition of chemotactic activity when RANTES were used as chemoattractant. Conversely, MVC induced a significant reduction of MIP-1β-induced chemotaxis, and this inhibition was dose-dependent ($P < 0{\cdot}05$ for all concentrations). A significant inhibition of chemotatic activity of MO in response to fMLP was found only when cells were treated with 1 and 10 μM of MVC ($P = 0{\cdot}008$ and 0·005, respectively). When MCP-1 was used as chemoattractant a significant inhibition of chemotaxis at all concentrations of MVC was found ($P < 0{\cdot}05$ for all) (Table 1).

Analysis of MO and DC chemotaxis following MVC treatment

Adherent monocytes were differentiated into MO and MDC, and the effect of MVC was tested. When MO were assessed, MVC affected chemotactic activity in response to all tested stimuli (Table 1). RANTES-induced chemotaxis was inhibited significantly by MVC only at concentrations of 1 and 10 μM ($P = 0{\cdot}03$ and 0·03, respectively). When migration of MO was assessed in response to MIP-1β, a significant inhibition was found at all MVC concentrations used ($P = 0{\cdot}001$). Similarly, fMLP-induced chemotaxis of MO was inhibited significantly by MVC in a dose-dependent manner ($P < 0{\cdot}001$). Finally, MCP-1-induced chemotaxis was inhibited at all concentrations of the drug, with a slight dose-dependent effect ($P < 0{\cdot}05$ for all) (Table 1).

When MDC chemotaxis was tested, MVC *in vitro* treatment induced a significant reduction of cell migration towards RANTES, MIP-1β, fMLP and MCP-1. RANTES-induced chemotaxis was decreased significantly by 0·1 μM, 1 μM and 10 μM of MVC (69% ± 6, 68% ± 6 and 72% ± 5 of the control, respectively; $P < 0{\cdot}05$ for all concentrations) (Fig. 1a). MIP-1β-induced chemotaxis of MDC was of 57% (±9), 54% (±9) and 45% (±12) of the control after treatment with 0·1 μM, 1 μM and 10 μM of MVC, respectively ($P < 0{\cdot}001$ for all three concentrations) (Fig. 1b). MVC inhibited fMLP-induced chemotaxis of MDC in a dose-dependent manner (53% ± 28, 37% ± 19 and 33% ± 17 of

Table 1. *In vitro* effect of CCR5 antagonist maraviroc (MVC) on chemotaxis of monocytes and macrophages.

Cells	MVC (μM)	RANTES-induced chemotaxis (%)	*P*	MIP-1β induced chemotaxis (%)	*P*	fMLP-induced chemotaxis (%)	*P*	MCP-1-induced chemotaxis (%)	*P*
Monocytes	0·1	95 ± 12	0·2	47 ± 12	< 0·05	67 ± 13	0·2	53 ± 11	< 0·05
	1	97 ± 12	0·2	33 ± 9	< 0·05	62 ± 14	0·008	56 ± 10	< 0·05
	10	90 ± 16	0·2	23 ± 7	< 0·05	60 ± 12	0·005	31 ± 9	< 0·05
Macrophages	0·1	76 ± 17	0·11	57 ± 9	0·001	59 ± 33	< 0·001	50 ± 18	< 0·05
	1	63 ± 3	0·03	56 ± 8	0·001	32 ± 23	< 0·001	66 ± 21	< 0·05
	10	61 ± 2	0·03	45 ± 6	0·001	8 ± 5	< 0·001	43 ± 15	< 0·05

Control chemotaxis was set at 100% and MVC treatments represent the percentage of control. Data are expressed as mean ± standard deviation of six independent experiments. *P*-value was calculated in comparison with control. RANTES: regulated upon activation normal T cell expressed and secreted; MIP: macrophage inflammatory protein; fLMP: formyl-methionyl-leucyl-phenylalanine.

© 2011 The Authors
Clinical and Experimental Immunology © 2011 British Society for Immunology, *Clinical and Experimental Immunology*

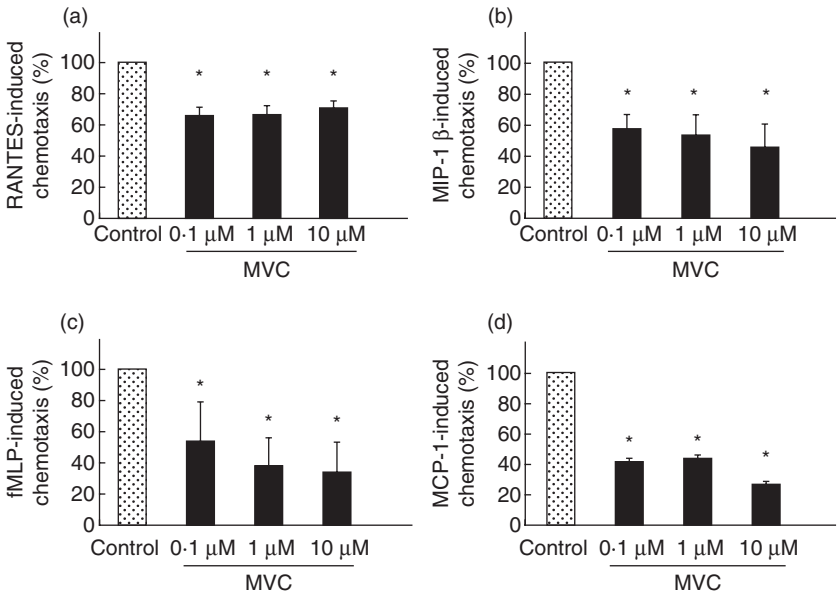

Fig. 1. Inhibitory effect of CCR5 antagonist maraviroc (MVC) on chemotaxis of monocyte-derived dendritic cells (MDC). MDC were treated with MVC (0·1, 1 and 10 μM) for 18 h and chemotactic activity was assessed by flow cytometric analysis. A significant reduction of MDC chemotaxis was seen towards regulated upon activation normal T cell expressed and secreted (RANTES) (a), macrophage inflammatory protein (MIP)-1β (b), formyl-methionyl-leucyl-phenylalanine (fMLP) (c) and monocyte chemotactic protein (MCP)-1 (d) at all drug concentrations used. Data were expressed as mean ± standard deviation of six independent experiments. Control chemotaxis was set at 100% and MVC treatments represented as the percentage of control (cells incubated with medium alone). Asterisks indicate a significant decrease in MDC chemotaxis *versus* control ($P < 0·05$).

the control after treatment with 0·1 μM, 1 μM and 10 μM of MVC, respectively ($P < 0·001$ for all three concentrations) (Fig. 1c).

Finally, MCP-1-induced chemotaxis of MDC was of 50% (±8), 66% (±11) and 43% (±10) of the control after treatment with 0·1 μM, 1 μM and 10 μM of MVC, respectively ($P < 0·005$ for all) (Fig. 1d).

A representative experiment of MDC chemotactic activity measured by Boyden's chamber method and Diff-Quik staining of filters is illustrated in Fig. 2.

Viability, cell phenotype and chemoattractant receptor expression

In another set of experiments, cell viability and phenotype (CD14 for monocytes, MO and CD1a for MDC) and expression of chemoattractant receptors CCR1, CCR4, CCR5 and FPR expression were investigated. We found no alteration in viability and phenotype in cells treated with MVC (data not shown). Moreover, treatment with different concentrations of MVC did not modulate CCR1, CCR4, CCR5 and FPR expression in monocytes, MO and MDC. The median of MFI in six independent experiments is reported in Table 2.

Discussion

Recent lines of evidence suggest that MVC, the first CCR5 antagonist approved in clinical practice for treatment of HIV infection, exhibit additional immunological effects beyond the pure anti-HIV inhibitory activity [10,11]. Given the central role of CCR5 in inflammation and cellular recruitment at the site of infection, analysis of the effect of CCR5 antagonists on cell migration may represent an area of active investigation [12]. In a recent paper, we demonstrated that PBMCs from HIV-infected patients exhibited diminished migratory responses toward fMLP after initiation of an antiretroviral regimen containing MVC [13]. In order to investigate if this phenomenon could be related to a direct effect of the drug, we analysed cell chemotactic activity after *in vitro* treatment with MVC. We found that MVC exhibited the ability to inhibit the chemotactic activity of PBMCs in response to fMLP and to CCR5-binding chemokine RANTES.

In the present study, we have investigated further the *in vitro* immunological effect of MVC by assessing the migratory capacity of APC, including monocytes, MO and MDC. We demonstrate for the first time that monocytes, MO and MDC have a different chemotactic response after *in vitro* treatment with MVC. In particular, the effect on chemotactic activity seems to be related to drug concentration as well as to substances used as chemoattractants.

MIP-1β, RANTES, MCP-1 and fMLP are important stimuli for both anti-infective response and inflammation [14,15]. MIP-1β is the natural ligand of CCR5 and cannot use other chemokine receptors. RANTES utilizes several receptors to induce chemotaxis, such as CCR1, 3, 4 and 5. Conversely, fMLP is a bacteria formyl peptide that regulates cellular trafficking and recognizes human FPR which is expressed in several cells, such as neutrophils, monocytes, MO and DC. Cross-talk between CCR5 expression and fMLP was described in monocytes, suggesting attenuation of cell responses to CCR5 ligands and inhibition of HIV-envelope glycoprotein-mediated fusion and infection of cells expressing CD4, CCR5 and FPR [16]. The same phenomenon was also found in DC [17].

We also analysed the effect of MVC on MCP-1-mediated chemotaxis. An increasing amount of evidence shows a close link between activated monocyte recruitment, MCP-1 release and HIV pathogenesis, especially in acquired immune deficiency syndrome (AIDS) patients suffering

© 2011 The Authors

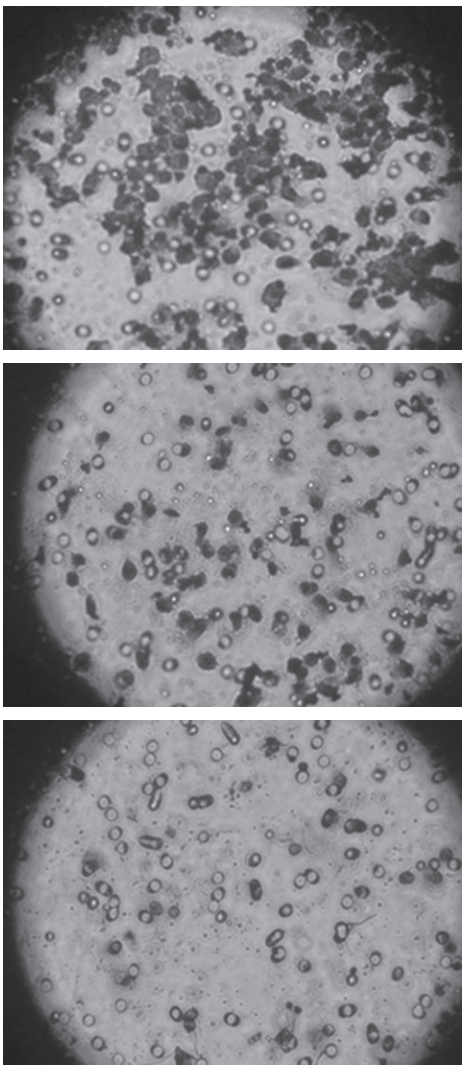

Fig. 2. Chemotactic activity of monocyte-derived dendritic cells (MDC) from one representative experiment after *in vitro* treatment with maraviroc (MVC). The MDC chemotactic activity was measured with the 8 μm pore size Transwell system using Boyden's method with blind-well chambers and DiffQuik staining of the filter. Cells were pretreated with MVC at concentrations of 0·1, 1 and 10 μM. In this representative experiment, the synthetic peptide formyl-methionyl-leucyl-phenylalanine (fMLP) (10^{-5} M) was used as chemoattractant.

from HIV-associated dementia [18]. It is important to study if MVC is able to inhibit migration of APCs towards CCL2/MCP-1 (a CCR2b ligand), because in cells co-expressing CCR5 and CCR2b, CCR5-specific ligands are able to prevent MCP-1 binding to its receptor. In fact, CCR5 and CCR2 are closely related and cross-competition between the two receptors has been found previously [19].

First of all, when we tested the effect of MVC on MIP-1β- and MCP-1-induced migration, our findings showed that the CCR5 antagonist compound was able to inhibit chemotaxis of monocytes, MO and MDC at all concentrations used. Chemotaxis towards RANTES, and fMLP was not inhibited by MVC at concentrations which were compatible with those achieved *in vivo* in the serum of treated subjects (0·1 μM). Cell chemotaxis was inhibited only when higher concentrations of the drug were used.

In HIV-infected patients, circulating MO and DC are often activated and this state of activation could be responsible for recirculation, inflammation and viral dissemination in the tissue [20,21]. Activated mature cells harvest HIV infectious particles and could transmit infection to $CD4^+$ T cells in the tissue [22]. Blockade of CCR5 could promote both the reduction of target cells for viral replication and the recruitment of activated T cells to inflamed lymphoid tissue. The anti-chemotactic activity of CCR5 antagonist MVC could have beneficial effects on HIV infection by blocking the migration of infected APCs into various tissues, such as brain, liver and lung. Moreover, it is known that activated MO and DC play a central role in the pathogenesis of atherosclerotic process, which now represents one of the major causes of morbidity and mortality of HIV-infected patients [22]. Inflamed plaques contain MO and subsets of fully matured and activated DC ($CD11c^+$), regulating the adaptive and innate immune system during the atherosclerotic process and plaque destabilization [23]. The accumulation of MO and DC in the atheroma and the relative depletion in the circulation [24] could stimulate both T cell recruitment and activation and may facilitate the release of chemokines, cytokines and other inflammatory mediators which are involved in the development and progression of HIV-associated atherosclerosis. Targeting CCR5 by MVC could have a double therapeutic effect in HIV-associated atherosclosis: blocking HIV entry into heart tissue via CCR5 and down-regulation of the accumulation of inflammatory cells in the atheroma. Moreover, the down-regulation of MCP-1-mediated chemotaxis induced by MVC could play a beneficial role in preventing the spread of HIV to the brain.

It is also known that both subsets of circulating myeloid DC (mDC) and plasmacytoid DC (pDC) are defective in HIV infection, especially because of homing in lymphoid organ and tissue [25,26]. After exposure to virions and HIV-infected cells, mDC and pDC up-regulate both tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and activation and migration markers, such as CD83 and CCR7, and acquire a killer-cytotoxic activity [27,28]. These cells down-regulate CXCR4 and CCR5 and become less susceptible to HIV infection; however, they are more active as proinflammatory cells by inducing apoptosis in infected and uninfected CD4 T cells and by producing cytokines such as interferon (IFN)-α and TNF-α. Our experiments suggest that MCV could inhibit chemotaxis, especially on these activated DC which are usually present during HIV infection. The anti-chemotactic activity of CCR5 antagonist could have also potential therapeutic implications for the management of inflammatory conditions other than HIV. The proposed mechanism of CCR5

© 2011 The Authors
Clinical and Experimental Immunology © 2011 British Society for Immunology, *Clinical and Experimental Immunology*

Table 2. *In vitro* effect of CCR5 antagonist maraviroc (MVC) on the expression of chemoactractant receptors.

Cells	MVC (μM)	CCR1 (MFI)	CCR4 (MFI)	CCR5 (MFI)	FPR (MFI)
Monocytes	0	369	351	421	157
	0·1	381	332	410	161
	1	366	317	402	168
	10	375	314	413	168
Macrophages	0	468	301	487	251
	0·1	528	364	471	243
	1	454	295	491	241
	10	573	297	495	248
Dendritic cells	0	603	335	607	383
	0·1	591	319	610	370
	1	589	320	620	390
	10	577	314	602	425

Data were expressed as median value of mean fluorescence intensity (MFI) in six independent experiments. No significant differences between MVC-treated and -untreated cells ($P > 0{\cdot}05$ for all).

antagonists in the treatment of rheumatoid arthritis involves inhibition of cell migration, a key pathway in the inflammatory process of the disease. In a mouse model of experimental autoimmune myocarditis (EAM) CCR5 was found to be important in the induction of the disease, and inhibition of CCR5 with monoclonal antibody reduced the severity of myocarditis significantly [29]. A critical issue associated with the block of cellular migration induced by CCR5 antagonist is a potential risk for treated patients of developing infectious complications. In effect, the reduced migratory capacity of MO and DC after pharmacological inhibition of CCR5 could impair the innate immune response against pathogens by blocking APC accumulation and activation at sites of microbial or antigenic challenge. Subjects homozygous for CCR5Δ32 who do not express CCR5 have a higher susceptibility to some infections, such as West Nile virus [30]. Moreover, studies from CCR5 knock-out mice demonstrated an increased morbidity and mortality after certain microbial challenges (Listeria, Cryptococcus, Toxoplasma, parainfluenza and influenza viruses) [31]. However, in the present *in vitro* study, the pharmacological blockade of CCR5 by MVC used at therapeutic concentrations does not seem to interfere with physiological recruitment of APC, such as monocytes, immature MO and DC. Moreover, clinical trials of MVC attest to its safety in the treatment of HIV-infected patients and no evidence of increase in infectious complications has been reported as yet.

The pathways involved in the down-regulation of MO and MDC chemotactic activity after *in vitro* treatment with MVC are not clear. MVC may lead to structural alterations in the chemokine receptor binding site and may induce long-lasting biochemical changes that impair the ability of specific chemokines receptor to work appropriately. The study of chemotactic receptor expression on cell surface as well as the measurement of cell calcium flux could contribute to a clearer understanding of the mechanisms of the MVC anti-chemotactic effect. In our study, we have shown that treatment with MVC did not induce any changes in CCR5, FPR, CCR1 and CCR4 expression in monocytes, MO and MDC. In addition, the analysis of MVC anti-chemotactic effect repeated in HIV-infected MO and MDC could be important to reproduce situations closer to those present in HIV-infected patients. Conversely, in previously *ex-vivo* experiments, we have shown that the chemotactic activity of HIV-infected PBMCs towards both RANTES and fMLP was inhibited significantly by MVC treatment [13]. However, further studies are needed to understand more clearly the mechanism underlying this inhibitory phenomenon exerted *in vitro* by maraviroc.

In conclusion, these findings suggest that CCR5 antagonist MVC is able to inhibit *in vitro* the migration of innate immune cells by mechanisms which could be independent from the pure anti-HIV effect. The drug might have a potential role in the down-regulation of HIV-associated chronic inflammation by blocking the recirculation and trafficking of mature MO and DC. Considering the increasing use of MVC in patients with HIV infection, further studies should be encouraged to understand the immunological consequences of CCR5 blockade in innate immune cells.

Acknowledgements

This study was supported by grants from the Health Ministry of Italy-ISS (AIDS project 2009–10).

Disclosure

None of the authors has any conflict of interests with the subject matter or materials discussed in the manuscript.

References

1 Medzhitov R, Janeway C Jr. Innate immunity. N Engl J Med 2000; **343**:338–44.

© 2011 The Authors

2 Luster AD. The role of chemokines in linking innate and adaptive immune response. Curr Opin Immunol 2002; **14**:129–35.
3 Lederman MM, Nicholson AP, Cho M *et al*. Biology and CCR5 and its role in HIV infection and treatment. JAMA 2006; **296**:815–26.
4 Deng H, Liu R, Ellmeier W *et al*. Identification of a major co-receptor for primary isolates of HIV-1. Nature 1996; **381**:661–6.
5 Dragic T, Litwin V, Allaway GP *et al*. HIV-1 entry into CD41 cells is mediated by the chemokine receptor CC-CKR-5. Nature 1996; **381**:667–73.
6 MacArthur RD, Novak RM. Maraviroc: the first of a new class of antiretroviral agents. Clin Infect Dis 2008; **47**:236–41.
7 Soriano V, Geretti AM, Perno CF *et al*. Optimal use for maraviroc in clinical practice. AIDS 2008; **22**:2231–40.
8 Lieberman-Blum SS, Fung HB, Bandres JC. Maraviroc: a CCR5-receptor antagonist for the treatment of HIV-1 infection. Clin Ther 2008; **30**:1228–50.
9 Emmelkamp JM, Rockstroh JK. CCR5 antagonists: comparison of efficacy, side effects, pharmacokinetics and interactions – review of the literature. Eur J Med Res 2007; **12**:409–17.
10 Soriano V, Perno CF, Kaiser R *et al*. When and how to use maraviroc in HIV-infected patients. AIDS 2009; **23**:2377–885.
11 Corbeau P, Reynes J. CCR5 antagonism in HIV infection: ways, effects, and side effects. AIDS 2009; **23**:1931–43.
12 Alkhatib G. The biology of CCR5 and CXCR4. Curr Opin HIV AIDS 2009; **4**:96–103.
13 Rossi R, Lichtner M, Sauzullo I *et al*. Downregulation of leukocyte migration after treatment with CCR5 antagonist maraviroc. J Acquir Immune Defic Syndr 2010; **54**:e13–14.
14 Ahuja SK, Gao JL, Murphy PM. Chemokine receptors and molecular mimicry. Immunol Today 1994; **15**:281–7.
15 Yang D, Chen Q, Le Y *et al*. Differential regulation of formyl peptide receptor-like 1 expression during the differentiation of monocytes to dendritic cells and macrophages. J Immunol 2001; **166**:4092–8.
16 Shen W, Li B, Wetzel MA *et al*. Down-regulation of the chemokine receptor CCR5 by activation of chemotactic formyl peptide receptor in human monocytes. Blood 2000; **96**:2887–94.
17 Le Y, Wetzel MA, Shen W *et al*. Desensitization of chemokine receptor CCR5 in dendritic cells at the early stage of differentiation by activation of formyl peptide receptors. Clin Immunol 2001; **99**:365–72.
18 Dhillon NK, Williams R, Callen S, Zien C, Narayan O, Buch S. Roles of MCP-1 in development of HIV-dementia. Front Biosci 2008; **13**:3913–18.
19 El-Asmar L, Springael JY, Ballet S, Andrieu EU, Vassart G, Parmentier M. Evidence for negative binding cooperativity within CCR5-CCR2b heterodimers. Mol Pharmacol 2005; **67**:460–9.
20 Jones GJ, Watera C, Patterson S. Comparative loss and maturation of peripheral blood dendritic cell subpopulations in African and non-African HIV-1-infected patients. AIDS 2001; **15**:1657–63.
21 Coleman CM, Wu L. HIV interactions with monocytes and dendritic cells: viral latency and reservoirs. Retrovirology 2009; **6**:143–54.
22 Marin B, Thiébaut R, Bucher HC *et al*. Non-AIDS-defining deaths and immunodeficiency in the era of combination antiretroviral therapy. AIDS 2009; **23**:1743–53.
23 Soehnlein O, Weber C. Myeloid cells in atherosclerosis: initiators and decision shapers. Semin Immunopathol 2009; **31**:35–47.
24 Lichtner M, Cuomo MR, Rossi R *et al*. Increased carotid intima media thickness is associated with depletion of circulating myeloid dendritic cells in HIV-infected patients on suppressive antiretroviral treatment. Atherosclerosis 2009; **204**:e1–3.
25 Pacanowski J, Kahi S, Baillet M *et al*. Reduced blood CD123+ (lymphoid) and CD11c+ (myeloid) dendritic cell numbers in primary HIV-1 infection. Blood 2001; **98**:3016–21.
26 Lichtner M, Rossi R, Rizza MC *et al*. Plasmacytoid dendritic cells count in antiretroviral-treated patients is predictive of HIV load control independent of CD4+ T-cell count. Curr HIV Res 2008; **6**:19–27.
27 Hardy AW, Graham DR, Shearer GM *et al*. HIV turns plasmacytoid dendritic cells (pDC) into TRAIL-expressing killer pDC and down-regulates HIV coreceptors by Toll-like receptor 7-induced IFN-alpha. Proc Natl Acad Sci USA 2007; **104**:17453–8.
28 Lichtner M, Marañón C, Vidalain PO *et al*. HIV type 1-infected dendritic cells induce apoptotic death in infected and uninfected primary CD4 T lymphocytes. AIDS Res Hum Retroviruses 2004; **20**:175–82.
29 Gong X, Feng H, Zhang S *et al*. Increased expression of CCR5 in experimental autoimmune myocarditis and reduced severity induced by anti-CCR5 monoclonal antibody. J Mol Cell Cardiol 2007; **42**:781–91.
30 Lim JK, Louie CY, Glaser C *et al*. Genetic deficiency of chemokine receptor CCR5 is a strong risk factor for symptomatic West Nile virus infection: a meta-analysis of 4 cohorts in the US epidemic. J Infect Dis 2008; **197**:262–5.
31 Telenti A. Safety concerns about CCR5 as an antiviral target. Curr Opin HIV AIDS 2009; **4**:131–5.

© 2011 The Authors
Clinical and Experimental Immunology © 2011 British Society for Immunology, *Clinical and Experimental Immunology*